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THE INTERNAL PHENOMENA OF REPRODUCTION IN DROSOPHILA.

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The present paper is limited to a description of the internal phenomena of reproduction in the vinegar fly *Drosophila melanogaster,* comprising under this title the ejaculation of the sperm by the male, its storage in the seminal receptacles of the female, and the discharge of the spermatozoa from the latter, at the time of the fertilization of the eggs.

The work was carried out in the Marine Biological Laboratory at Woods Hole during the summer of 1919 at the suggestion of Professor T. H. Morgan, to whom I am indebted for advice and helpful criticism, also for valuable information gathered by him in his attempts to bring out artificial fertilization in this fiy. An obstacle in the study of the experimental fertilization in *Drosophila* is the high sensitiveness of the spermatozoa to the action of fluids foreign to the body; they are easily injured and killed when placed in mixtures which do not exert noxious effects on the sperm of other animals, unless the spermatozoa are kept in such fluids for a long time. Although I tried repeatedly to eliminate this obstacle by the use of several called "indifferent" fluids, it was impossible to keep the spermatozoa active beyond a few minutes.

During these experiments I gathered interesting data on the normal reproduction of *Drosophila,* in which, owing to the small size and transparent condition of the internal generative organs,

it was possible to observe the contents of the extirpated uterus and seminal receptacles of the fertilized fly in the living condition. These organs were kept under direct observation for some time, and the entrance of the spermatozoa into the receptacles and the position of the egg within the uterus were seen directly instead of having to be inferred from series of sections taken at different stages in these processes. Sections have been used as a means of checking up the results obtained by direct observation.

Drosophila has thus provided almost unique material for the study of the internal phenomena preceding normal fertilization in insects, for besides the facts just mentioned, the mating of a considerable number of flies can be easily brought about at any desired time.

I. TECHNIQUE.

The wild stock of *Drosophila melanogaster* was used throughout the work. Virgin females isolated shortly after hatching were mated to males of the same generation three or four days after isolation. The matings were accurately timed in every case.

The flies were either etherized or killed by a preserving fluid while in copulation or as soon as this was finished. In the first case the observations were made on the living genitalia, these organs being dissected out under a binocular microscope in a drop of Ringer's fluid or normal salt solution, after which they could be observed with a high power microscope. In order to follow the path of the spermatozoa into the receptacles and observe the position of the egg within the uterus, no cover glass was added, and in this way any pressure which might interfere with the first process or crush the egg was avoided. If a cover glass is used and gently pressed down with a needle, the ventral receptacle, normally coiled, will straighten and its contents may be easily observed under high power, the spermatozoa being readily detected when present.

The flies which were preserved were embedded in paraffin and cut into sections. The specimens were always dropped alive in the following fluid, after the formula of W. Docters van Leeuwen:[1]

[1] *Zool. Anz.*, Bd. 32, 1908.

Picric acid (1 p.c. sol. in abs. alc.)	6	c.c.
Chloroform	1	c.c.
40 per cent. formalin	1	c.c.
Glacial acetic acid	0.5	c.c.

This fluid kills instantly and penetrates very well; but the preservation obtained is poor for cytological purposes yet good enough for histological study. The abdomens of the flies were cut off from the rest of the body while in the fixative, then transferred to 95 per cent. alcohol and cleared with cedar oil, after dehydration with absolute alcohol. Sagittal sections were used almost exclusively and were stained with Delafield's hematoxylin and eosin.

2. The Morphology of the Internal Generative Organs.

The internal generative organs of *Drosophila* are much like those in other flies. In the female they show certain characteristics absent or feebly developed in other diptera.[2]

Male.

The testes (Fig. 1, *t*) are two tightly convoluted tubes of orange or yellow color, which communicate with the vas deferens (*d*) by means of rather short, paired ducts, the vasa efferentia (*v*). The latter are swollen in their initial portion and probably store the ripe spermatozoa before they are discharged into the vas deferens. The vas deferens is a long duct, much swollen in its anterior section, tapering gradually towards its termination at the side of the ejaculatory sac (*s*). Its walls are highly contractile.

The ejaculatory sac is a curious structure which deserves special mention since it plays an important rôle in the ejaculation of the sperm. It acts as a pump and drives the sperm through a narrow ejaculatory duct (*e*) which has stiff walls lined internally with a tough cuticle. The sac can be regarded as an outpocketing of the ejaculatory duct, since it is also lined internally with a cuticle, but its cavity has been cut off from the lumen of the duct which is in communication with the vas deferens through an en-

[2] So far as is known to the writer, the occurrence of a ventral seminal receptacle has never been mentioned in any of the Diptera. Cf. Berlese, 1909.

larged portion (Fig. 2, *c*) placed against the proximal surface of the sac. The whole organ may be compared with two kidney-shaped hollow bodies fused along a sagittal plane, their cavities being confluent. The single cavity thus arising is lined with a thin cuticle produced by the columnar epithelium (*w*) which

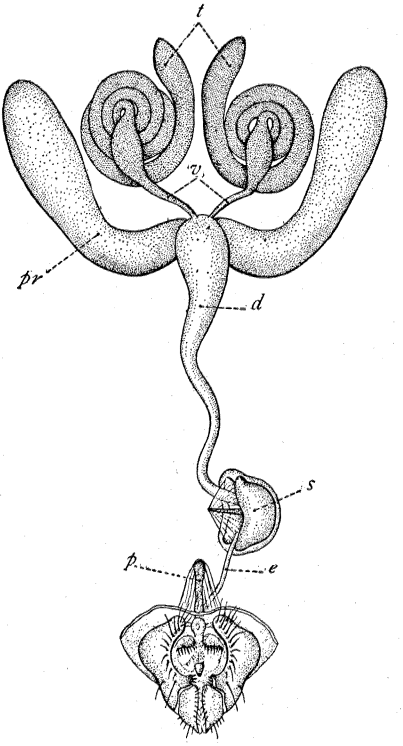

FIG. 1. Male generative organs of *Drosophila melanogaster*, ventral view. *d*, vas deferens; *e*, ejaculatory duct; *p*, penis; *pr*, paragonia; *s*, ejaculatory sac; *t*, testes; *v*, vasa efferentia.

forms the walls of the organ, and contains a highly refractive, thick fluid (*f*) secreted by the epithelium. This fluid is scanty in young flies, a small amount being present in those just hatched. It is entirely separated from the outside since the cavity in which it is contained has no outlet. In the proximal surface of the sac the cuticle secreted by the epithelium is thickened to form a plate bearing a rod-shaped sclerite (*s*) which projects freely into the body cavity. The sclerite is connected with the outer walls of

the sac by means of powerful muscle fibers (*m*) which form a cone, the axis being determined by the rod.

The enlarged portion (*c*) connecting the vas deferens with the ejaculatory duct borders the rod-shaped sclerite forming a crescent and therefore is not pierced by it, as inspection of Fig. 2 might lead one to suppose. As the sclerite is a projection from the plate mentioned above, the latter forms a considerable part of the wall of the cavity contained in this enlarged portion, which collects the sperm coming from the vas deferens.

The mechanism that drives the sperm through the narrow ejaculatory duct appears to be very simple but highly efficient.

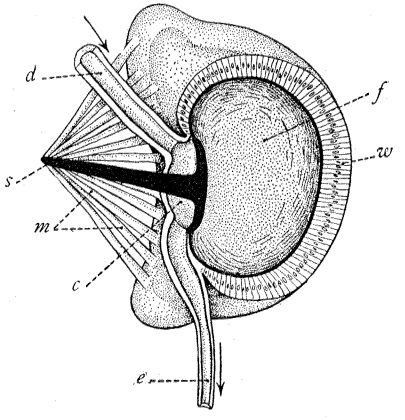

FIG. 2. Diagram of the ejaculatory sac based on the study of sections and on the observation of the fresh organ. The sac has been represented as cut along the sagittal plane. *c*, cavity connecting the vas deferens, *d*, with the ejaculatory duct, *e*; *f*, fluid contained within the sac; *m*, muscle fibers; *s*, sclerite; *w*, epithelial wall of the sac.

Upon contraction of the muscle fibers connecting the sclerite with the walls of the sac, the thick plate at the base of the rod-shaped sclerite sinks into the cavity of the sac, compressing the fluid therein. At the same time the portion connecting the vas deferens with the ejaculatory duct is expanded, the partial vacuum thus produced causing the sperm in the former to flow into its cavity. The relaxation of the muscle fibers aided by the elasticity of the fluid contained in the sac brings the plate of the sclerite to its original position, and as a result the sperm in the cavity is driven out under pressure through the ejaculatory duct.

The return of the sperm into the vas deferens seems to be prevented by the partial or total closure of the orifice of communication.

The thick fluid within the ejaculatory sac is not poured into the duct; in flies killed during copulation or shortly after, no loss of fluid could be observed. On the other hand, the spermatozoa do not penetrate the cavity of the ejaculatory sac, as proved by the study of sections of flies killed during ejaculation. In these

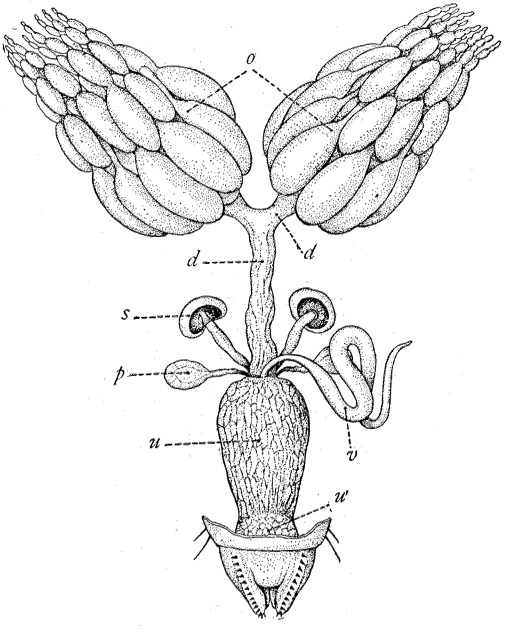

FIG. 3. Female generative organs, ventral view. *d*, oviducts; *d'*, azygos oviduct; *o*, ovaries; *p*, parovaria; *s*, spermathecae; *u*, uterus; *u'*, vaginal portion of the uterus; *v*, ventral receptacle, represented as uncoiled in this figure.

the sac appears filled with a coagulated homogeneous fluid, while in the portion of the vas deferens and ejaculatory duct nearest to the organ several bundles of spermatozoa could be seen.

The only accessory glands present in the male are the so-called paragonia (Fig. 1, *pr*), also termed seminal vesicles in spite of the fact that spermatozoa are rarely or never found in them. They are two large sacs which open into the vas deferens, their openings being placed a little below those of the vasa efferentia.

They contain a dense, sticky fluid in which float abundant refractive granules of unequal size. This fluid is mixed with the spermatozoa at their entrance into the vas deferens and forms the liquid part of the ejaculate.

Female.

The ovaries (Fig. 3, *o*) are connected by means of short paired oviducts (*d*) with a single azygos oviduct (*d'*) which opens in the anterior end of the uterus (*u*). The latter is a sac with highly muscular walls, lined internally by an epithelium of cubical

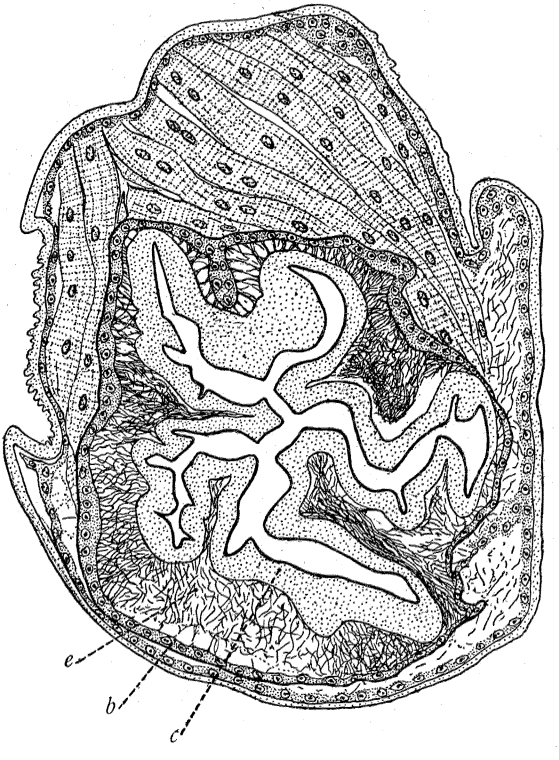

FIG. 4. Cross section of the vagina, showing the irregular lumen and the papillæ in which the epithelium is thrown. *b*, basal portion of the epithelium containing the nuclei; *e*, fibrillar portion; *c*, cuticle.

cells, which secretes a thin cuticle. Two distinct regions can be recognized in this organ: a distal region or uterus proper (Figs. 3 and 9, *u*) and a proximal region or vagina (*u'*) in which the

epithelium is highly modified, the thin cuticular lining being much thicker and thrown into a few very distinct ridges (Fig. 4, *c*). The anterior end of the uterus forms a pouch (Fig. 5, *a*) capable of great distension; its walls are thrown into irregular foldings which in sections appear as papillæ. The oviduct opens dorsad to this pouch by a wide opening (Fig. 5, *d*).

The female of *Drosophila* differs from that of most of the other flies in the presence of an unpaired seminal receptacle

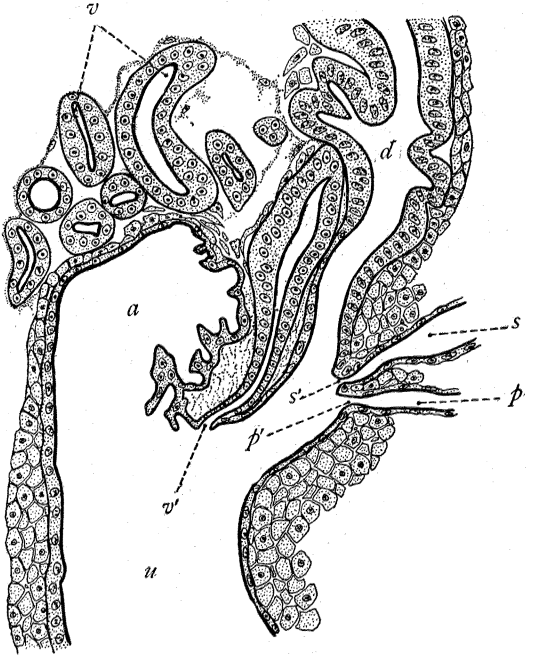

FIG. 5. Sagittal section of the anterior portion of the uterus in a virgin fly. *a*, anterior pouch; *d*, oviduct; *p*, duct of one of the parovaria with *p'*, its opening in the dorsal wall of the uterus; *s*, duct of one of the spermathecæ, with *s'*, its opening into the uterus; *u*, uterine cavity; *v*, ventral receptacle with *v'*, its opening into the uterus.

placed in the ventral surface of the uterus (Fig. 3, *v*) and by the existence of two smaller receptacles or spermathecæ (*s*). Occasionally three spermathecæ occur and this condition seems to be normal in many of the diptera (Berlese, 1909).

The spermathecæ (Figs. 3 and 9, *s*) open into the dorsal wall

of the anterior portion of the uterus by means of two narrow openings, placed close to each other. They are mushroom-shaped bodies whose terminal cavity is lined by a hard cuticle of brown color (Fig. 6, c') secreted by the epithelium (e) forming the walls of the organ. They are connected with the uterus by narrow ducts which on account of circular ridges present in their

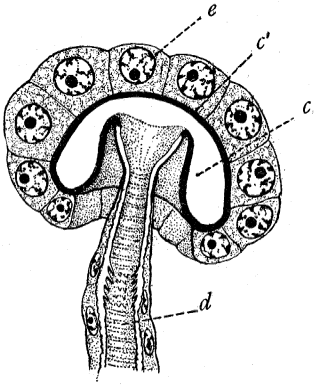

FIG. 6. Diagram of a spermatheca. c, cavity lined with the brown cuticle c'; d, duct of the spermatheca; e, epithelium.

cuticle closely resemble tracheæ. The circular ridges of the distal portion of each duct bear processes resembling stiff hairs which are directed towards the openings of the ducts (d) into the uterus.

The ventral seminal receptacle (Figs. 3 and 9, v) is a long convoluted tube which opens into the anterior portion of the uterus, immediately dorsad to the anterior pouch (Fig. 5, v'), between the latter and the oviduct. The lumen of this tube is very narrow in its proximal portion, gradually expanding and becoming narrow again in the distal or free portion of the organ. In the normal position the tube is tightly coiled and is placed at the ventral surface of the uterus, immediately above the anterior pouch. The lumen is lined with a cuticle produced by an epithelium of cubical cells.

The size of the ventral receptacle varies a good deal according to the species; in *Drosophila obscura* it is shorter and wide, while in *D. virilis* it attains an enormous length.

A sagittal section of the anterior portion of the uterus (Fig. 5) shows that the opening of the ventral receptacle (v') is placed a

little nearer the vagina than the openings of the spermathecæ (*s'*), and this position seems to have some relation to the entrance of the spermatozoa into the receptacles and their discharge at the time of fertilization.

Besides the organs described there are two other organs (Figs. 3 and 9, *p*) which correspond in structure with the so-called parovaria in other forms (Lowne, 1893). These organs are obviously glands; they possess a cavity filled with a colloidal fluid containing minute refractive granules. They are connected with the anterior portion of the uterus by means of ducts, which open near the orifices of the spermathecæ (Fig. 5, *p'*). The walls of the parovaria are made up of large cells which contain round nuclei and a peculiar structure, described in *Calliphora* by Lowne and erroneously interpreted by this observer as a future egg.

In virgin females the wall of the uterus collapses, the lumen being quite narrow and irregular.

3. The Ejaculation of the Sperm and its Entrance into the Seminal Receptacles.

The observation of the living uterus and the study of sections of flies killed at different periods during mating showed that the ejaculation of the sperm usually takes place about nine or ten minutes after the beginning of copulation.

Female flies killed immediately after the ejaculation of the sperm showed that, although most of the spermatozoa seemed to be motionless at this time, they occasionally exhibit faint undulatory movements. If they do not move freely it may be because the mass of spermatozoa is subjected to the pressure exerted by the walls of the uterus or possibly to a considerable extent because of the viscid condition of the fluid portion of the ejaculate. But it is evident that they are at least potentially active, as shown by their behavior in later stages.

It was not possible to study the movements of the spermatozoa when outside of the uterus, the fluids foreign to the body causing at first a decrease in their activity, followed by their death. I have dissected flies in several fluids such as tap and distilled water, normal saline solution (0.5 per cent.), Ringer's fluid (cold-

blooded), 1 per cent. solution of potassium citrate,[1] and 1 per thousand solution of sodium hydroxide. All of these fluids injure the spermatozoa more or less rapidly, and fail to keep them alive beyond two or three minutes. On acount of the small size of the flies it was not possible to obtain body plasma in sufficient amount to tease the uterus in it and observe the behavior of the spermatozoa.

The spermatozoa do not enter the seminal receptacles immediately after ejaculation has taken place. There is a pause which usually lasts two or three minutes or even more, during which the cavity of the uterus is packed with spermatozoa while the seminal receptacles are entirely empty. This peculiarity was observed once, and again in the uterus of flies killed after ten or eleven minutes of copulation. These results are consistent with those obtained by the study of sections of flies preserved at the same stage, the spermatozoa being crowded in the uterine cavity, while the receptacles and their ducts do not show any spermatozoön whatsoever.

The occurrence of this pause suggests that some change must take place that causes the spermatozoa to swim actively, for the swimming movements are very conspicuous when they enter the receptacles in contrast to their almost passive condition while in the uterus. This fact points to an influence on the part of the parovaria which are the only accessory glands in the female genitalia whose function is obscure. Their bearing on the formation of the shell of the egg is very doubtful, the latter being completely formed when entering the uterus. Lowne thought that the parovaria in the blow-fly furnish the very young oöcytes; he reached this striking conclusion on account of the presence of certain structures in the cytoplasm of their cells which he compared with the oöcyte of mammals since they are surrounded with a thin layer resembling the zona pellucida of the mammalian egg. As already mentioned these curious structures are also present in the parovaria of *Drosophila*. Lowne's interpretation is not warranted by any fact, other than a superficial resemblance, the two

[1] According to Koltzoff, 1908, the spermatozoa of the rhinoceros beetle, *Oryctes nasicornis*, may be kept alive in a 2 per cent. aqueous solution of potassium citrate for a week; this observer thinks that their death is caused by the growth of bacteria in the fluid rather than through exhaustion.

structures being essentially of a different kind. It seems unnecessary to insist here upon this point. The glandular nature of the parovaria is the more probable interpretation.

However, it was not possible to ascertain the kind of influence exerted by these glands on the spermatozoa, since all the experiments to solve this problem had to be carried out in a fluid foreign to the body, which, as stated above, kills the spermatozoa. Under such conditions it is hardly possible that the secretion of the parovaria may cause any response in already injured elements. While the secretion of the parovaria may activate the spermatozoa it is still possible that they are concerned only with the dissolution of the thick fluid portion of the ejaculate, thus removing one of the obstacles which prevent free motion.

The entrance of the spermatozoa into the receptacles has been repeatedly observed by the writer in the living genitalia, for several minutes at a time. Owing to the impermeability of the walls of the receptacles and their ducts, the noxious effects of the fluids in which the dissections were carried out were not felt at once. The ventral receptacle is the first to receive the sperm. Since the amount of sperm ejaculated usually exceeds the quantity which can be stored in the receptacles it is common to see spermatozoa which have made their way into the oviduct. As will be pointed out later, on account of the position of the egg when passing through the latter, it is safe to assume that such spermatozoa do not play any rôle in the fertilization of the first eggs coming into the uterus, which very often, and for reasons not well known, are sterile.

At the time of their entrance into the ventral receptacle, the spermatozoa in the vicinity of the openings of the parovaria, namely, those crowded in the anterior portion of the uterus, begin to show strong movements and make their way into the receptacle by swimming actively, a stream of them being detected along the coils of this organ. When they reach the tip of the latter the swimming movements cease, to be replaced by undulatory movements, which decrease in intensity as new spermatozoa arrive. After two to five minutes the whole lumen of the receptacle is packed with sperms.

When the ventral receptacle is filled with sperms, the same

process is repeated in the spermathecæ. Here it is still easier to follow their entrance, for the ducts of these receptacles are very thin and transparent. In no case were spermatozoa observed entering these receptacles before the ventral receptacle was filled with spermatozoa. This might be explained by the position of the orifice leading to the latter, which, as already stated, is placed a little posterior to the openings of the spermathecæ, and perhaps also by the partial closure of the orifices leading to these organs.

The spermatozoa contained in the seminal receptacles form bundles; in the ventral receptacle all the heads are directed toward the tip or free end of the organ. In the spermathecæ the bundles are concentric.

Once within the receptacles the spermatozoa cease to swim, showing only undulatory movements; in this condition they can live for many days, even weeks.

5. The Egg and its Entrance into the Uterus.

The eggs of *Drosophila melanogaster* are very small, elliptical in shape and bear at one end a pair of diverging appendages

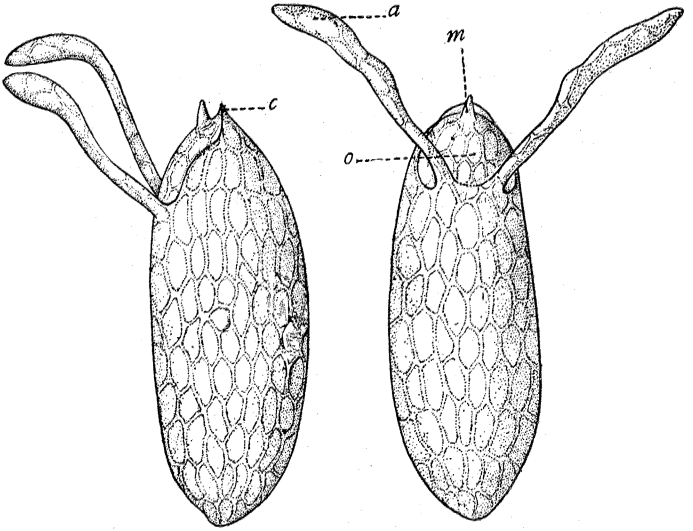

FIG. 7. Dorsal and lateral views of the egg of *D. melanogaster*. *a*, appendages; *c*, plate formed by the edge of *o*, transparent area resembling an operculum; *m*, micropyle cone.

whose function is not well known. They probably prevent the sinking of the egg when laid on the fermented fruit, thus allowing the hatching larvæ to creep over the food without being asphyxiated. These appendages (Fig. 7, *a*) arise from one of the surfaces of the egg. For descriptive purposes I will call it the dorsal surface since it is applied against the dorsal wall of the uterus when the egg is within the latter.

The end of the egg nearest to the place from which the appendages arise bears a conical structure, the micropyle cone (*m*), which is pierced by an extremely narrow canal, the micropyle. Observed under higher power, the micropyle cone appears hollow (Fig. 8, *c*) with the cytoplasm of the egg (*p*) penetrating its

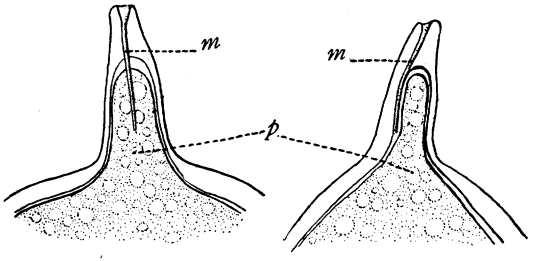

FIG. 8. Ventral and lateral views of the micropyle cone of *D. melanogaster* drawn from the fresh egg. *m*, micropyle; *p*, protoplasm of the egg penetrating into the micropyle cone.

interior. The canal or micropyle (*m*) goes from the tip of the cone towards its base and is placed on the side continuous with the ventral surface of the egg. The pole of the egg bearing the micropyle is always directed toward the anterior end of the animal, either when in the ovary (Fig. 3) or when in the uterus (Fig. 9).

In the portion of the dorsal surface in front of the appendages there is an area more transparent than the rest of the chorion, which could be termed the operculum in the event of the escape of the hatching larva through this region of the egg. However, this does not always happen, the anterior end of the egg splitting rather irregularly and further ventrally than this.[1] The lateral borders of the transparent area form a ridge which appears as a thin plate at the apex of the egg, passing ventrally the micropyle cone.

[1] I am indebted for this observation to Dr. A. H. Sturtevant.

The outer chorion of the egg shows hexagonal sculpturing produced by the hardening of the much flattened follicle cells surrounding the oöcyte during the growth period. This pattern is a little less developed in the transparent region described above (Fig. 7, *o*).

The passage of the eggs through the oviduct takes place very

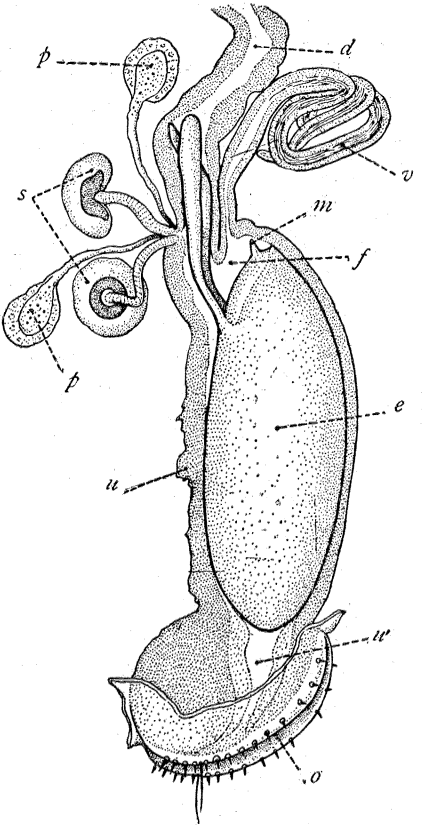

FIG. 9. Lateral view of the uterus containing an egg; this figure was outlined with the camera lucida, the genitalia being in the fresh condition. *e*, egg with the appendages within the oviduct, *d*; *f*, fertilization cavity; *m*, micropyle cone of the egg; *o*, vaginal orifice; *p*, parovaria; *s*, spermathecæ; *u*, uterus with *u'*, its vaginal portion; *v*, ventral receptacle, containing spermatozoa.

quickly if we judge from the considerable number of egg-laying females dissected in which no eggs were present in this duct. The actual entrance of an egg into the oviduct was observed only

once. Waves of contraction were noticeable in one of the ovaries, extending from its tip to the base and caused apparently by the contraction of muscle fibers placed in the thin connective tissue layer enveloping the organ. At the same time the oviduct expanded and contracted rhythmically thus bringing one of the eggs lying near the entrance of one of the paired oviducts into its lumen. The contractions were strong enough to push the egg into the oviduct, and probably the process would have gone further had it not been for the exhaustion brought about by the artificial environment in which the organs were placed. These contractions were very conspicuous in the ovaries of all the flies dissected, whether laying eggs or not, and may be explained by the action of the fluid in which dissection was carried out, or by the action of free oxygen on the muscle fibers of the ovary and oviduct, which take the place of the nervous stimulus in the living fly.

The orientation of the egg, when passing through the oviduct, is the same as that in the ovary, the micropyle cone being directed toward the latter. The egg is about three times as wide as the oviduct, which is, therefore, considerably distended when the egg is passing through it.

These conditions make impossible the fertilization of the eggs in the oviduct. Since the micropyle is not facing the opening of the latter into the uterus, and since the egg is larger than the oviduct, any spermatozoa, if present, would be pressed aside by the passing egg in the event of their penetration into the oviduct.

The size of the uterus is so well adapted to that of the egg that the latter occupies almost entirely its cavity (Fig. 9), the appendages remaining within the oviduct (*d*), thus blocking the way of any spermatozoa that might enter into the latter when the sperm is poured out of the seminal receptacles during fertilization. A small space (*f*) is left in the anterior portion of the uterus, between the walls of the anterior pouch and the surface of the egg, and in this space swim the spermatozoa coming from the seminal receptacles.

The position of the egg within the uterus is constantly the same in all the flies observed, including several females of *Drosophila obscura*. The dorsal surface of the egg, where the appendages

arise, is applied against the dorsal wall of the uterus, while the opposite surface lies against the ventral wall. The anterior end of the egg, bearing the micropyle is lodged in the anterior pouch of the uterus; the micropyle cone is directed toward the opening of the ventral receptacle, and in some cases seems to be almost in contact with it.

Owing to the size of the egg no space is left between its chorion and the wall of the uterus; both of these come in close contact thus preventing the spermatozoa from being diverted from the anterior pole of the egg, where the penetration of a spermatozoön takes place.

6. The External Phenomena of Fertilization.

Under this heading I will consider all the phenomena leading to the penetration of the egg by a spermatozoön, the discharge of the sperm from the ventral receptacle and spermathecæ, and the way in which the last process is accomplished. The actual penetration of the egg by the spermatozoön was never observed owing to the lack of transparency in the walls of the anterior portion of the uterus, which prevents the detection of isolated spermatozoa, and also to the fact that it is very difficult to ascertain the moment in which an egg enters into the cavity of the uterus.

A series of experiments showed that in all probability the first spermatozoa used in fertilization are those stored in the ventral receptacle, those kept in the spermathecæ being used later, when the contents of the former are exhausted. When planning these experiments it was thought that the best way to ascertain the facts would be to allow several females to be fertilized and examine them at definite periods after which the condition of the ventral receptacle and spermathecæ could be observed by dissection. The time chosen for the first lot of flies was seven days. The food was changed daily, and the eggs laid counted every day. There is, however, an important obstacle, namely, that the rate of laying is variable in different females. But this proved to be very helpful, for, instead of the ventral receptacle being found empty in all the flies, a rough relation between the number of eggs laid and the amount of sperm stored in this receptacle was found. Ten flies were used in this experiment; two of them died

in the first four days, the others laying eggs continuously during the seven days. The flies belonged to the same generation, were isolated on the same day, the isolation period prior to mating being three days. The number of eggs layed was 470, 336, 335, 238, 221, 206, 200 and 117. In the female which laid 470 eggs only a few spermatozoa could be detected in the ventral receptacle. In the female which laid the smallest number of eggs (117) about two thirds of the ventral receptacle was filled with spermatozoa. The amount of sperm present in the ventral receptacle of the other flies could not be estimated accurately, but decreased noticeably as the number of eggs laid increased. Inspection of the spermathecæ in all of the eight flies showed the presence of abundant spermatozoa closely packed; no spermatozoa were detected along the ducts of these organs. Additional dissections of flies killed from twelve to fourteen days after fertilized proved in all cases the correctness of this view.

As the ventral receptacle begins to empty its spermatozoa it may be seen that the latter are turning around so as to have their heads directed toward the opening of the receptacle into the uterus. This rotation was very conspicuous in most of the flies dissected, the spermatozoa being scattered in the proximal portion of the receptacle without definite orientation.

The bulk of the sperm does not go to the immediate vicinity of the opening of the ventral receptacle into the uterus when their discharge begins, but remains in the free end of the receptacle, only a few traversing the empty space which separates the latter from the orifice, to be poured into the anterior portion of the uterus. The inspection of the initial portion of the receptacle showed a few spermatozoa swimming towards the uterus when this contains an egg; the portion of the receptacle near the opening is so narrow that only a few spermatozoa can pass at a time.

Owing to the position of the micropyle in the egg within the uterus it was thought that the spermatozoa stored in the spermathecæ might be unable to fertilize any eggs, since the orifices leading to these organs are placed in the dorsal wall of the uterus and the appendages of the egg come to lie between these orifices and the micropyle. Some experiments were carried out in order to ascertain this important point. Ten flies were simultaneously

mated to males of the same generation, then isolated in vials, the food being changed daily. When the first sterile eggs were laid by each fly it was dissected and the conditions of the spermathecæ carefully observed. The following table shows the result of this experiment, in which the number of eggs laid each day was not recorded.

TABLE I.

Flies.	No. of Days Laying.	Condition of the Spermathecæ.	Condition of the Ventral Receptacle.
1	20	Sperm present	Empty
2	12	Empty	"
3	22	"	"
4	21	"	"
5	20	"	"
6	22	"	"
7	20	"	"
8	20	"	"
9	20	"	Sperm present
10	22	"	Empty

With the exception of fly No. 1, which laid some sterile eggs while the spermathecæ still retained spermatozoa, all the other flies showed no spermatozoa at all. Whether the sterile eggs laid by such a fly had received spermatozoa or not is a point impossible to settle. It is possible that the sterility in this case was related to intrinsic factors in the egg, rather than to a failure in the discharge of the spermatozoa from the receptacles. The ventral receptacle appeared empty in all of the flies except specimen No. 9 which showed a quite irregular behavior in the rate of laying. I do not know the causes of the retention of spermatozoa in the ventral receptacle of this fly, which appeared entirely normal in other respects.

These results show that all the spermatozoa, whether they are stored in the ventral receptacle or in the spermathecæ, are used in fertilization. The presence of foldings in the epithelium lining the anterior pouch of the uterus probably plays an important rôle in the fertilization of the eggs by spermatozoa coming from the spermathecæ, since such outgrowths of the epithelium form deep grooves along which the spermatozoa may progress until they reach the empty space left between the egg and the walls of the uterus.

Although, as stated above, the presence of sperm in the spermathecæ of flies which have been laying eggs for several days suggests that the first spermatozoa used in fertilization are those stored in the ventral receptacle, which appears empty or containing few spermatozoa, still the same conditions may appear if the amount of sperm received by the spermathecæ surpasses by far that stored in the ventral receptacle. In other words, the largest amount of sperm will last longer, provided that spermatozoa are simultaneously set free by all the seminal receptacles.

Further experiments were carried out with the hope of clearing up this point. These experiments consisted in allowing fertilized females to lay eggs during a period of six days, and then cross them with a mutant which would enable me to recognize the hybrids in the F_1, without need of testing the offspring. The mutant used was the bar-eyed male. Seven flies were used in the experiment, which covered a period of eighteen days. The food was changed daily and placed in separate vials to allow the eggs to develop.

These experiments showed the following facts: (1) When the number of flies produced during the first six days is low, the offspring following fertilization by the bar male consists of hybrid and normal females, the males being normal in all the cases. This result can be accounted for by the presence of spermatozoa from the first copulation in the ventral receptacle where they become mixed up with those received during copulation with the bar male. (2) When, on the contrary, the number of flies produced during the first six days is high, only hybrid females are produced during the first days following fertilization by the bar male. That is to say, the ventral receptacle was already empty of sperm. It was then filled by the sperm ejaculated by the bar male, and, therefore, only hybrid females were produced. In such a case mixed offspring appear after a few days, when the spermatozoa stored in the spermathecæ begin to be used.

The results obtained in the second case have been graphically shown in the curve of Fig. 10, plotted after the data obtained in a female which had produced a large number of flies during the

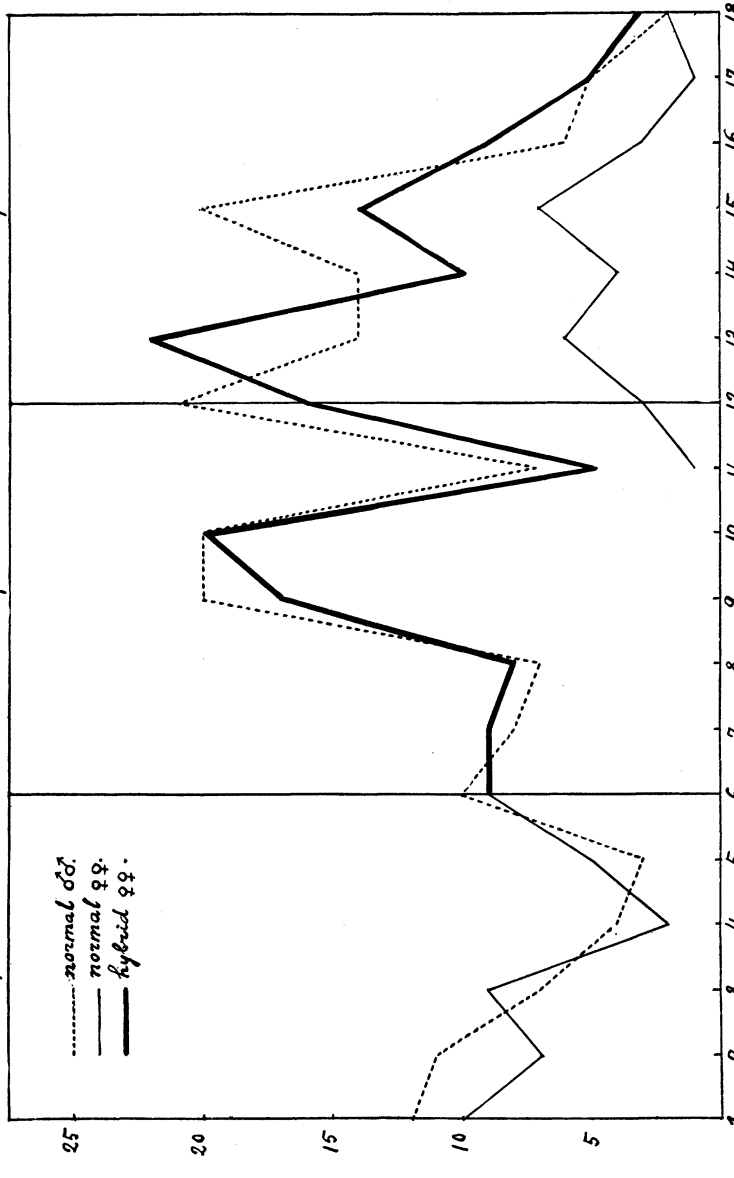

FIG. 10. Curve representing the results of crossing a normal, fertilized female with a male of the bar-eyed mutant. During the second period only hybrid females are produced, showing that the spermatozoa kept in the ventral receptacle at that time are those from the bar male.

first period.[1] During the first five days after mating to the bar-eyed male only hybrid females were produced; at the sixth day normal females began to appear. Fortunately this fly was killed at a critical moment, when fertilization of the eggs was about to stop. Dissection showed that the ventral receptacle and one of the spermathecæ were empty, while a few active spermatozoa could be seen moving along the duct of the other spermatheca.

It is interesting to note that the number of normal females produced after crossing with the bar male is smaller than that of the hybrid females. This peculiarity, which stands out clearly in all the experiments, is due to the fact that the number of flies hatched is higher when the parents belong to different mutant races.

An important point in the mechanism of the discharge of the sperm from the seminal receptacles is the influence exerted by the egg on the spermatozoa. Whether or not the latter are attracted by the egg is a point which could not be determined by the writer. Although such an attraction, due to a chemotactic stimulus, is possible, we must keep in mind that the same result may be attained by reflexes set up when the egg enters the uterus, the release of the spermatozoa taking place upon dilatation of the orifices leading to the seminal receptacles. The behavior of the spermatozoa in sterile flies would throw some light on this problem, showing at least whether their discharge is related to the entrance of an egg into the uterus. Such sterile flies are said to be rather common in the inbred stocks of *Drosophila melanogaster* (Hyde, '14); they do not lay eggs, probably on account of the abnormal condition of their oviducts. If there is any relation between the laying of eggs and the discharge of the spermatozoa, such flies not laying any should keep the spermatozoa in their receptacles; otherwise the latter would appear empty after some time. Some sterile flies which appeared in the bar-eyed stock were given to me by Dr. Morgan, in the hope that this point could be settled. Unfortunately such flies could not be mated, the males giving up courtship after a short time. Very often a male

[1] The number of flies hatched must not be taken as a fair representation of the number of eggs laid. Both males and females may die in relatively large numbers during development, either as the result of intrinsic lethal factors or of inappropriate environmental conditions. To this is added the sterility of some eggs, especially during the first days following copulation.

which would readily mate, having been kept isolated in a vial for three or four days, becomes discouraged after smelling the posterior end of the female, where normally a white matter acting as a cause of sexual excitement protrudes (Sturtevant, '15). Sterile flies usually die after a few days, their abdomens attaining considerable distension. In the only female dissected the paired oviducts lacked a lumen; some four or five eggs had fallen into the body cavity by rupture of the ovarian wall, and were entirely degenerated, the cytoplasm appearing in irregular clumps. All the seminal receptacles, which like the uterus were normal, did not contain sperm.

Conclusions.

1. In the internal genitalia of *Drosophila melanogaster* there are only two spermathecæ and a ventral seminal receptacle in the form of a long, convoluted tube which opens proximally into the anterior portion of the uterus, ventral to the oviduct.

2. The spermatozoa after ejaculation into the uterus during copulation appear almost motionless, only a few showing faint undulatory movements. The ejaculation of the sperm by the male is greatly aided through the action of the ejaculatory sac, an organ which acts as a pump, driving the sperm at a high pressure through the narrow ejaculatory duct.

3. After ejaculation of the sperm there is a pause which lasts two or three minutes, during which the spermatozoa do not show active movements. Then they begin to swim actively to enter the seminal receptacles. There seems to be an activation of the spermatozoa at this moment on the part of the parovaria, two glandular structures connected with the anterior portion of the uterus by means of narrow ducts.

4. The ventral receptacle is the first to receive the sperm, which is also stored in the spermathecæ. The spermatozoa form bundles when in the receptacles. In the ventral receptacle all the heads are directed toward the distal end of this organ. The bundles are concentric in the spermathecæ.

5. The position of the egg within the uterus is constant; the surface bearing a pair of divergent appendages is applied against the dorsal wall of the uterus. The anterior end of the egg with the micropyle is lodged in the anterior portion of the uterus, with

the micropyle cone directed toward the opening of the ventral receptacle. The appendages of the egg remain within the oviduct thus preventing the entrance of spermatozoa into this organ. Fertilization of the eggs takes place in the uterus.

6. The observation of the extirpated internal genitalia in fertilized flies which had been laying eggs for several days and some experiments show that in all probability the first spermatozoa used in fertilization are those stored in the ventral receptacle, while the spermatozoa kept in the spermathecæ are used later.

7. The influence of the secretion of the parovaria on the spermatozoa and the behavior of the latter when in the vicinity of the egg could not be ascertained on account of the injurious effects of the fluids in which these experiments were carried, on the spermatozoa, which are very sensitive to fluids foreign to the body.

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